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Simple and rapid LC-MS/MS method for the absolute determination of cetuximab in human serum using an immobilized trypsin



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ABSTRACT

Proteomic approaches using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) without an immunopurification technique have not been applied to the determination of serum cetuximab. This study developed a simple and rapid LC-MS/MS method for the absolute determination of cetuximab in human serum and applied it to clinical settings. Surrogate peptides derived from cetuximab digests were selected using a Fourier transform mass spectrometer. Reduced-alkylated serum cetuximab without immunopurification was digested for 20 minutes using immobilized trypsin, and the digestion products were purified by solid-phase extraction. The LC-MS/MS was run in positive ion multiple reaction monitoring mode. This method was applied to the determination of serum samples in head and neck cancer patients treated with cetuximab. The chromatographic run time was 10 minutes and no peaks interfering with surrogate peptides in serum digestion products were observed. The calibration curve of absolute cetuximab in serum was linear over the concentration range of 4–200 $\mu\text{g}/\text{mL}$. The lower limit of quantification of cetuximab in human serum was 4 $\mu\text{g}/\text{mL}$. The intra-assay and inter-assay precision and accuracy were less than 13.2% and 88.0–100.7%, respectively. The serum concentration range of cetuximab was 19–140 $\mu\text{g}/\text{mL}$ in patients. The serum cetuximab concentrations in LC-MS/MS were correlated with those in ELISA ($r = 0.899$, $P < 0.01$) and the mean bias was 1.5% in cancer patients. In conclusion, the present simple and rapid method with acceptable analytical performance can be helpful for evaluating the absolute concentration of serum cetuximab in clinical settings.